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Occurrence of an exotic earthworm (*Amynthas agrestis*) in undisturbed soils of the southern Appalachian Mountains, USA

Mac A. Callaham, Jr.[1,*], Paul F. Hendrix[2] and Ross J. Phillips[1]

[1] USDA Forest Service, Forestry Sciences Laboratory, Athens, GA, 30602, USA
[2] Institute of Ecology, University of Georgia, Athens, GA, 30602, USA

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Summary

This study documents the occurrence of an aggressive invasive earthworm species in undisturbed forest soils of the southern Appalachian Mountains of northern Georgia, USA. Earthworms were sorted from samples collected in pitfall traps that had been set in mature, mesic oak-hickory forests in remote, high elevation, locations across northern Georgia. Specimens were continuously collected in these traps over the course of the summer and autumn of 1993, and more than 600 earthworms were collected from 35 different trapping sites. There were at least 9 different earthworm taxa collected during the study including three species not native to North American soils (*Amynthas agrestis*, *Octolasion tyrtaeum*, and *O. cyaneum*). The majority of earthworms collected in the study were *A. agrestis*. Because large numbers of *A. agrestis* were trapped at a single site, we made measurements of individuals in an attempt to examine the reproductive status of *A. agrestis* at that site over time. Small numbers of *A. agrestis* were trapped in July and August, peak abundance occurred in September, and there was a decline in abundance through November to zero trapped in December. Sexually mature adults were first trapped in small numbers in late August, but made up 80 % of the total population by mid September, and 100 % of the population in October and November.

Key words: *Amynthas agrestis*, biological invasion, Appalachian, soil fauna, exotic species, earthworms

Introduction

Introduced species dominate the earthworm fauna in most soils of North America (Gates 1976). This is particularly true in soils that have undergone some disturbance such as agricultural or other development. A general feature is that native earthworm species are found in relatively undisturbed soils whereas introduced species are more likely to be encountered in disturbed soils (Hendrix 1995; Kalisz & Dotson 1989). Because surveys in or near agricultural, or otherwise disturbed, soils may bias collections toward disturbance-tolerant and non-native European, Asian and African species, sampling in remote areas is needed to gauge the extent of intact native communities and the degree to which exotic species have dispersed into

*E-mail corresponding author: mcallaham@fs.fed.us

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Appendix 1. List of the new sampling locations presented in this study

T14, Mateur, edge of wet ditch, sticky clay, 17.10.1989. **T15**, Saf Saf, steppe with water trickle, sticky clay, 17.10.1989. **T16**, Jeradou, dry oued with oleanders and few trees, clay, 4.11.1989. **T17**, Zriba, S aspect of Dj. Zaghouan, pine wood on hillside, 450 m, sandy soil, 5.11.1989. **T18**, N aspect of Dj. Zaghouan, pine plantation and mixed wood on hillside, 500 m, humic soil, 5.10.1989. **A56**, Ain El Assel, *Q. suber* wood with eucalyptus and acacia, red sandy soil, 18.10.1989. **A57**, ESE of Annaba, Lac des Oiseaux, Oleaster and acacia on sandy soil, 18.10.1989. **A58**, Plage de Seraidi, degraded maquis, meager arenaceous soil on micaschist, 19.10.1989. **A59**, Seraidi, *Q. suber* forest, red soil rather humid, 19.10.1989. **A60**, Seraidi, *Q. afares* forest on steep hillside, 500 m, rich litter and humus, 19.10.1989. **A61**, Ain Barbar, mixed oak forest with a stream, 800 m, 20.10.1989. **A62**, SW of El Aouana, cultivated fields, arenaceous soil on granite, 21.10.1989. **A63**, summit of Pic des Singes, maquis on red soil on granite, 21.10.1989. **A64**, S aspect of Dj. Babor, stream in mixed *Cedrus* wood on slope, 1250 m, limestone on schists, 22.10.1989. **A65**, S of Bordj-Bou-Arrerdj, steppe with thin bush, 1000 m, 23.10.1989. **A66**, Kalaa Beni-Hammad, Mt. Hodna, stream in gorge with oleanders, 1150 m, submerged mud, 24.10.1989. **A67**, Bou Saada, El Hamel, oasis in steppe with cultivated patches fed by a river, 800 m, submerged travertin, 25.10.1989. **A68**, Biskra, Oued El Abiod, date palm grove beside nearly dry oued, 26.10.1989. **A69**, Chedma, palm grove with olive trees, figs, water pools, wet clay with grass, 26.10.1989. **A70**, N of Barrage de Foum-El-Gherza, palm grove with olives, stony-gravely soil, 26.10.1989. **A71**, Arris, filling station, sandy soil near water pipe, 27.10.1989. **A72**, 8 km W of Arris, river bed in steppe with few juniper and pine, 28.10.1989. **A73**, Ain El Beida, steppe with low scrub on mountain pass, 1800 m, moist soil under stones, 29.10.1989. **A74**, Tazoult-Lambèse, shaded hillside with a few juniper and *Q. coccifera*, dry soil, 30.10.1989. **A75**, S aspect of Dj. Fortasse, spring and stream from limestone knoll, 1000 m, wet mud, 1.11.1989. **A76**, Tebessa, stream below pine forest on limestone slope, 1175 m, 2.11.1989. **A90**, Beni Abbès, cultivated land, clayey soil, 14.2.1995. **A91**, Husseyn Dey, cultivated land, clayey soil, 12.5.1996. **A92**, Jijel, cultivated land, podzol, 12.12.1995. **A93**, Tizi Ouzou, cultivated land, podzol, 11.12.1994. **A94**, Hammam-Righa Oued, grass on clayey soil, 12.11.1995. **A95**, Forêt de Hadjout, sandy soil, 15.11.1995. **A96**, Constantine, grass on clayey soil, 9.9.1995. **A97**, Blida, cultivated land, clayey soil, 22.11.1996. **A98**, Algiers, Hamma, grass on podzol, 15.11.1995. **A99**, Birtouta, cultivated land, clayey soil, 12.11.1995 and 9.12.1995. **A100**, El Arrach, cultivated land, clayey soil, 9.1.1995. **A101**, Ghardaia, palm grove, sandy soil, 12.4.1994. **A102**, Algiers, Ben Aknoun, cultivated land, clayey soil, 13.12.1995. **A103**, Sétif, cultivated land, podzol, 12.2.1996. **A104**, Baraki, cultivated land, podzol, 20.9.1996. **A105**, Oran, cultivated land, ferrugineous soil, 19.3.1996. **A106**, Miliana, grass on podzol, 15.10.1995. **A107**, Guelma, 9.10.1997. **A108**, Lac Tonga, El Aioun, 5.1.1995. **A109**, Lac des Oiseaux, 5.1.1995. **A110**, Algiers, Kouba, campus of the École Normale Supérieure, 10.1.1995. **M25**, Plain de Gareb, ditch fed by Oued Kert, 19.4.1986. **M26**, El-Arba-Taourit, mountain pass with pine plantation and cultivated fields, 1000 m, arid soil, 20.4.1986. **M27**, Targuist, slope with *Quercus coccifera* and pine plantation, stony soil, 20.4.1986. **M28**, 4 km W of Ketama, *Cedrus* wood and degraded mountain pasture, 1550 m, argilloschists, 21.4.1986. **M29**, 8 km W of Ketama, *Cedrus* wood with *Pyrus* near a fountain, stony humic soil and mosses, 21.4.1986. **M30**, 6 km W of Ketama, *Betula*, *Cedrus*, *Pyrus*, *Ilex*, poor soil on argilloschists, 21.4.1986. **M31**, Bab Basen, *Cedrus* wood with oaks, humus and bark, 21.4.1986. **M32**, Bab Berred-Bab Basen, *Cedrus* wood with oaks, 1650 m, brown soil on argilloschists, 22.4.1986. **M33**, 4 km W of Ketama, *Betula*, *Quercus*, *Cedrus* and pines, shallow soil on argilloschists, 22.4.1986. **M34**, Maison Forestale Bab Berred, *Quercus* wood adjacent to pines and poplars, humid soil, 1450 m, 23.4.1986. **M35**, Chechaouen, *Q. suber* and maquis, 900 m, litter and soil, 23.4.1986. **M36**, W of Oued Loukos, wet ditch at roadside, 350 m, 23.4.1986. **M37**, valley of Oued Loukos, mixed oak wood (*Q. rotundifolia*, *Q. suber*, *Q. faginea*) with maquis, 24.4.1986. **M38**, Ouezzane, under the bridge and in oak wood, sand and litter, 25.4.1986. **M39**, Col du Zeggota, cultivated fields, 25.4.1986. **M40**, Moulay-Idriss, *Q. coccifera* and cultivated fields at edge of Oued Kroumane, 700 m, 26.5.1986. **M41**, Jebel Zerhoun, stream among cultivated patches on calcareous rocks, 750–800 m, 27.4.1986. **M42**, Ifrane, stream at edge of *Q. rotundifolia* wood, 1550 m, 29.4.1986. **M43**, Foret de Jaba, WSW of Ifrane, oak wood on dry, red soil, 1550 m, 29.4.1986. **M44**, Ifrane Lake, mud, 30.4.1986. **M45**, Foret de Mamora, *Q. suber* and maquis, sandy soil, 1.5.1986. **M46**, N of Larache, *Q. suber* wood on dry, sandy soil, 180 m, 2.5.1986. **M47**, Aakba-Amra, *Q. suber* wood, red soil, 2.5.1986. **M48**, Asilah, pond with *Ranunculus*, mud, 2.5.1986. **M49**, Cap Spartel, bed of uadi, calcareous sand, 2.5.1986.

Appendix 2. List of the species recorded at the new locations

Criodrilus lacuum: A66. ***Hormogaster redii***: T15, T18. ***Eiseniella neapolitana***: A96. ***E. tetraedra***: A64, M29, M30, M33, M44. ***Eisenia fetida***: A75, A91, A93, A105, A107. ***E. parva***: A92, A97. ***E. xylophila***: A61, A103. ***Lumbricus friendi***: M34. ***L. rubellus***: A110. ***Proctodrilus antipai***: A56, A75. ***Helodrilus*** cfr. ***oculatus***: A97, A99. ***Allolobophora borellii***: A67, A95, A99. ***A. georgii***: A92, A93, A96. ***A. molleri* complex**: A66, A67, A108, A109, M34, M36–38, M40, M42–45, M49. ***A. rosea* complex**: T16, T18, A56, A63–67, A71, A73, A75, A91, A93, A94, A96–100, A109, A110, M25–35, M37, M39–41, M43, M45, M46. ***Nicodrilus* sp. "gigas"**: T14. ***Nicodrilus caliginosus* complex**: T15, T17, T18, A56, A57, A58, A61, A63–69, A71, A72, A75, A76, A90–93, A95–97, A99–102, A105, A106, A108, A109, M25–37, M39–41, M43–48. ***Murchieona minuscula***: A98, M35. ***Allolobophoridella eiseni***: M31, M32. ***Dendrobaena lusitana***: A98, M34, M35, M37. ***Octodrilus complanatus***: A93, A95–97, A99, A100, A102, A104, A105, A110, M43. ***O. maghrebinus***: A56, A59, A61, A64, A74, A92. ***Amynthas* sp.**: A93, A97, A105. ***Ocnerodrilus* sp.**: A67, A76. ***Microscolex dubius***: A62, A90, A91, A93, A96, A98, A103, A106, M35. ***M. phosphoreus***: A59, A60, A63, A68, A70, A91, A97, A98, A100, A110. ***Dichogaster* sp.**: A97, A99.

these areas. With a few exceptions (Kalisz & Dotson 1989; Reynolds et al. 1974; Reynolds 1978), sampling in remote, relatively undisturbed areas in the southern Appalachian mountains has only recently begun, and is expected to yield numerous previously unknown native species (James, personal communication). Information about interactions between native and non-native species is scarce, but this type of information is critical to understanding the potential consequences of ongoing biological invasions in North American soils. The existence of biological invasions in soil is largely overlooked because soil is an opaque medium, where the biota are inconspicuous (Hendrix & Bohlen 2002; Ehrenfeld & Scott 2001).

Materials and Methods

For five months during summer and autumn, 1993, researchers from the University of Georgia Museum of Natural History conducted an extensive pitfall trapping campaign in some of the most remote, high-elevation mesic oak-hickory forests in northern Georgia. Climate in these forests is subtropical humid, with annual rainfall of ~1800 mm, and mean annual temperatures of 13 °C. The objective of the overall study was to capture small mammals, amphibians, and reptiles. However, large numbers of invertebrates were also accidentally captured in the pitfalls, including earthworms. Pitfall trapping is not considered to

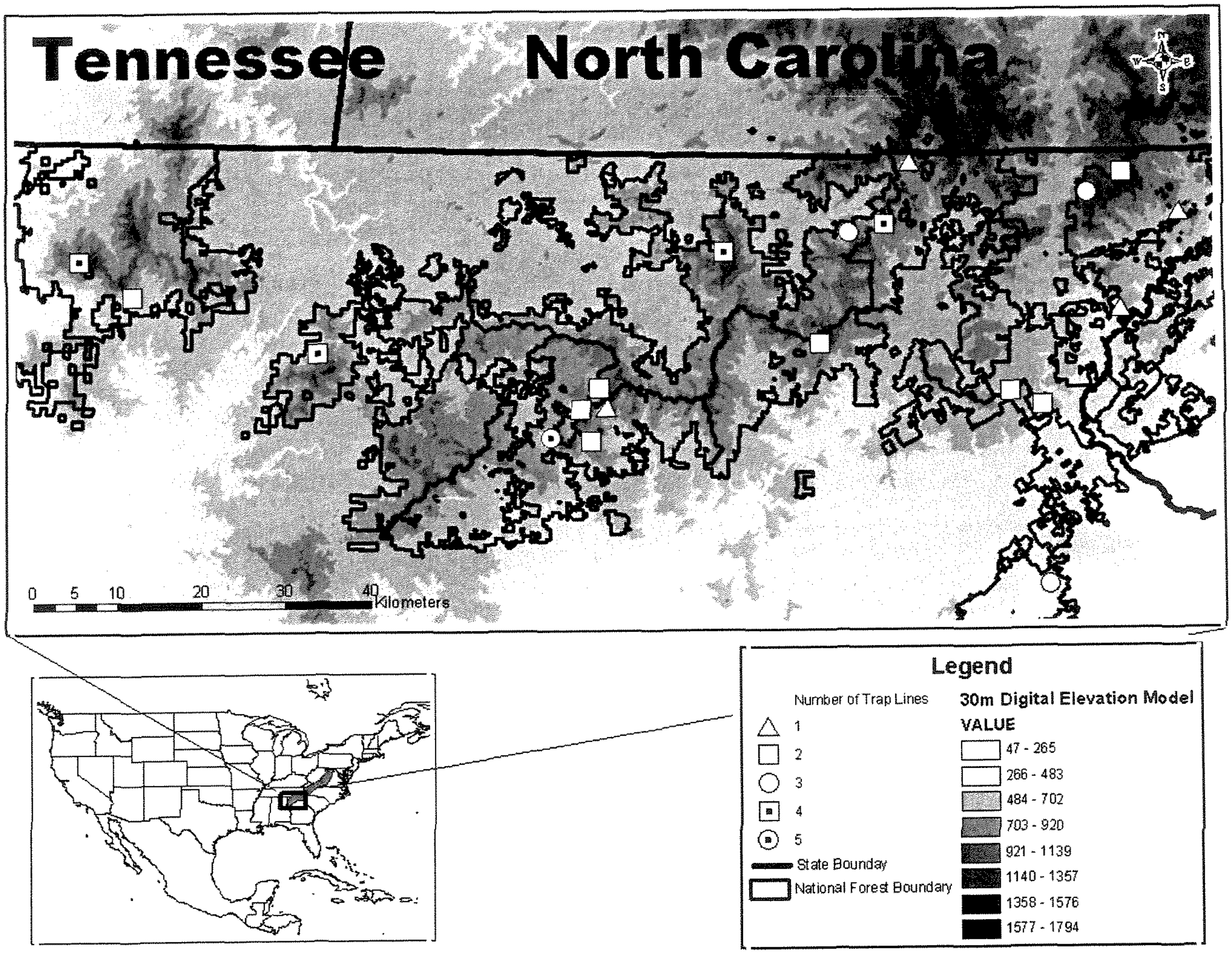

Fig. 1. Locations of pitfall trap lines in northern Georgia. Symbol shapes indicate trapping locations, see map legend for number of trap arrays at each location. Elevations are in meters

be a particularly efficient method for collection of earthworms, because captures of earthworms can be influenced by differences in climatic conditions, and differences in behavior of earthworms at different developmental stages. Also pitfall trapping is biased toward collection of epigeic worms, surface-feeding anecic worms, or individuals moving on the soil surface following rainfall. Thus, inferences made from collections in this study extend only to earthworms active on the soil surface, and can not address trends in the whole earthworm community. Nevertheless, the unintentional collection of several hundred earthworms in this study presented an opportunity to examine material from areas that had not previously been sampled.

Pitfall trapping sites consisted of 20–25 traps set in an array across an area of approximately 2 ha. There was a total of 50 arrays set across 20 locations (see Fig. 1 for locations and number of arrays at specific locations). Traps were set by burying ~1L cups flush to the soil surface, and partially filling with 5 % formalin. Traps were set near coarse woody debris, rock outcroppings or other forest floor materials likely to be used as cover by small vertebrates. Each array was checked, and traps emptied of specimens, approximately every two weeks throughout the study. Earthworms were separated from other specimens, counted, measured, and identified to species when possible and to genus or family if specimens were non-reproductive (using keys of Schwert 1990; James 1990; Reynolds 1978).

Results and Discussion

There were more than 600 earthworm specimens collected during the five-month pitfall trapping campaign. Earthworms were collected at 35 of the 50 trap arrays, and at 16 of the 20 sites. Greater than 75 % of the total number of earthworms collected were exotic species (Table 1). Another 20 % consisted of juvenile lumbricids that may have been native or exotic species (the family Lumbricidae is represented by both native and introduced species in the samples). In contrast, only 4 % of the earthworms collected were verifiably native North American species (Table 1). There were at least nine different earthworm taxa collected (Table 1). Native earthworms were represented by the lumbricid genera *Bimastos* and *Eisenoides*, the megascolecid genus *Diplocardia*, and by a single specimen of the genus *Komarekiona*.

Exotic species captured in pitfalls included the lumbricid genus *Octolasion*, and the megascolecid genus *Amynthas*. *Amynthas agrestis* (Goto and Hatai, 1899) made up 73 % of the total number of earthworms and 96 % of the exotic earthworms collected (Table 1). This species is believed to have been introduced to North American soils via materials imported from Japan (Gates 1982). This overwhelming abundance of an exotic species in these relatively undisturbed sites was surprising, particularly given the findings of Kalisz & Dotson (1989), where exotic earthworm species were encountered only in severely disturbed areas in the mountains of eastern Kentucky.

Table 1. Number of individuals from each earthworm group collected in pitfall traps from the southern Appalachian mountains July – November 1993. Also shown is the number of sites, out of the 50 total trapping sites, where each group occurred

Group	# collected	% of total	# of sites collected	% of sites occurred
Native earthworms				
Bimastos palustris	1	0.2	1	2
Bimastos zeteki	3	0.5	2	4
Bimastos spp.	5	0.8	5	10
Diplocardia spp.	12	1.9	7	14
Eisenoides carolinensis	1	0.2	1	2
Komarekiona sp.	1	0.2	1	2
Introduced earthworms				
Amynthas agrestis	464	73.0	11	22
Octolasion tyrtaeum	13	2.0	5	10
Octolasion cyaneum	7	1.1	5	10
Other earthworms				
Lumbricid juveniles	122	19.2	20	40
Unidentifiable	7	1.1	3	6

Note: Juvenile lumbricids could be either native or introduced. Unidentifiable specimens had deteriorated to such a degree that no determination could be made

Because *Amynthas* agrestis was collected in greater numbers than any other species, and because of its status as an aggressive invader, this species warranted further examination. One site in particular (on the slopes of Brasstown Bald, the highest elevation in Georgia, ~1450 m) yielded large numbers of *A. agrestis*, and we examined the demography of the specimens collected at this site in detail (Fig. 2). In the early phases of trapping (July and early August), only small juveniles were collected. A peak in abundance of these juvenile and sub-adult individuals was observed to be in late August (Fig. 2). In September, total abundance of *A. agrestis* dropped, but abundance of adults peaked. There were no juvenile or sub-adult individuals collected after September (Fig 2). Individuals reached reproductive maturity at a length of ~115 mm (formalin preserved length).

The patterns of abundance for juvenile and adult *A. agrestis* observed in this study are in general agreement with those presented for the species in Reynolds (1978). However, the occurrence of *A. agrestis* in the present study in high elevation, relatively undisturbed sites is in contrast to the collections of Reynolds (1978). Although Reynolds sampled in more than 1600 different locations in Tennessee, he encountered *A. agrestis* in only 17 locations, and of these 17, 8 are described as "dumps", and 9 collections were made in "fields, under logs". These habitat descriptions indicate severe disturbance, whereas our collections were made in generally undisturbed, mature forest stands.

The dominance of *A. agrestis* in the pitfall traps used in this study is likely attributable to their epigeic behavioral characteristics. This species is very active in the litter layer of the forest floor and should be more susceptible to collection by pitfall trap. However, it is notable that the native North American genus *Bimastos* is also generally epigeic, but was poorly represented in these collections relative to *A. agrestis*.

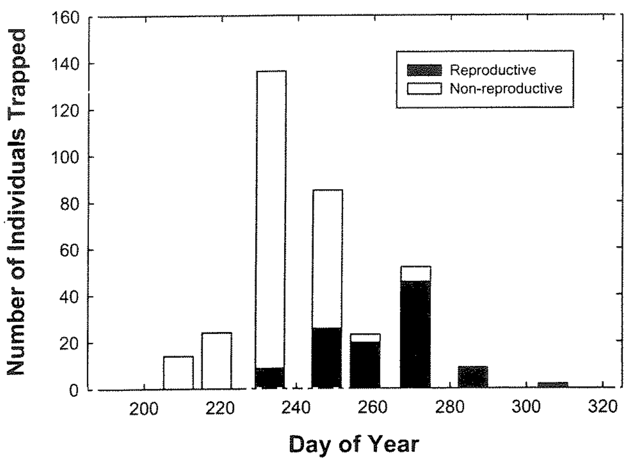

Fig. 2. Demographic characteristics of *Amynthas agrestis* trapped at one site (Brasstown Bald, trapline #2) over the course of the study. Number of juvenile and adult earthworms trapped at each date are shown. Open portion of bar indicates number of juvenile or sub-adult individuals, closed portion of bar indicates number of reproductive adults

Tandy (1969) reported that *Amynthas* spp. were purposely introduced to certain areas in Louisiana for establishment of bait populations "around the margins of lakes" (*cf.* Reynolds 1978). *Amynthas* spp. are also commonly sold as fish bait in northern Georgia, and many popular spots for trout fishing are located in the mountainous region of the state. Thus, the original introduction of *A. agrestis* to these remote areas may have been the result of fishermen discarding unused bait. Just a few individuals of *Amynthas* could be sufficient for establishment of new populations, as most species in the genus (occurring in North America) are parthenogenetic (Reynolds 1978).

This study documents the occurrence of *Amynthas* agrestis in habitats not previously reported for this species. These findings are of concern because remote, undisturbed locations are thought to be the last refugia for many native earthworm species in the southern Appalachian Mountains (Kalisz & Dotson 1989; Kalisz & Wood 1995). Furthermore, there is increasing evidence that *Amynthas* can significantly alter organic matter dynamics and forest floor structure in North American forests (Burtelow et al. 1998), and that exotic earthworm activity can negatively affect other organisms living in or on the forest floor (Gundale 2002). Understanding the life-history factors associated with establishment, development and spread of exotics such as A. agrestis is crucial for developing methods of control for these species. Our study should provide a base of information for such efforts.

Acknowledgements. This work is dedicated to the memory of Professor Joshua Laerm. Laerm's boundless enthusiasm for making zoological collections, and his recognition of the fact that there were significant numbers of earthworms being collected in his pitfall traps, are principally responsible for the eventual completion of this work. Lee Hartle of the Georgia Museum of Natural History is gratefully acknowledged for making Laerm's extensive notes and collections available for this study.

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